6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting,' and/or measuring, and/or monitoring zinc, its metabolites, and other biomarkers of exposure and effect to zinc. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

Zinc is ubiquitous in both the environment and the laboratory. Since many biological and environmental samples contain low levels of zinc, it is easy to contaminate the samples. While analyzing samples, it is imperative that special precautions be taken to avoid sample contamination in order to obtain accurate results and ensure the integrity of the sample. Blood collection tubes are potential sources of zinc contamination (Delves 1981). An example of failure to institute proper measures to control sample contamination, which led to inaccuracies in reported data, was described by Windom et al. (1991). Methods that can be used to avoid reporting erroneous results include interlaboratory data comparison (Galloway et al. 1983) or use of standard reference materials, such as certified SRM 1549 (nonfat powdered milk) available from the National Institute of Standards and Technology (Perry 1990).

6.1 BIOLOGICAL MATERIALS

Table 6-l lists the applicable analytical methods used for determining zinc in biological fluids and tissues.

Atomic absorption spectrometry (AAS) using a furnace atomizer is a common and simple laboratory technique. It has become the method of choice for zinc analysis in biological samples including bone, liver, hair, blood, and urine. It is an economical method for determining trace

TABLE 6-1. Analytical Methods for Determining Zinc in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Breath	Personal sampler cartridges	ICP; AES	0.6 μg/L	94–101	NIOSH 1984a (method 7300)
		AAS, flame	3 μg/sample	NR	NIOSH 1984a (method 7030)
Blood or tissue	Acid digestion with $HNO_3/HClO_4$, H_2SO_4, measure at 213.9 nm	ICP; AES	1 μg/100 g (blood); 0.2 μg/g (tissue)	103	NIOSH 1984a (method 8005)
Urine	Acid digestion of oxygen plasma ashing; extract with polydithiocarbamate resin; measure at 213.9 nm	ICP; AES	0.1 μg/sample	100	NIOSH 1984a (method 8310)
Semen	Microwave wet acid digestion	GFAAS	400 μg/L	96–104	Alvarado et al. 1991
Fingernails	Digest nail samples with concentrated nitric acid; heat at 65°C for 1 hour; cool and dilute with deionized water	AAS, graphite furnace	NR	NR	Sohler et al. 1976

TABLE 6-1. Analytical Methods for Determining Zinc in Biological Materials *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Liver	Acid digestion with mixtures of different acids; distill volatile elements	Radiochemical NAA	NR	98	Lievens et al. 1977
Liver	Homogenize sample with water; add HCl; shake; centrifuge; dilute	FAAS	40 µg/L	100	Luterotti et al. 1992
Muscle tissue	Mineralize sample in muffle furnace; dissolve in HNO_3	FIA	3 µg/L	NR	Fernandez et al. 1992b
Blood	Separate serum from blood by centrifugation; transfer a portion of serum into an ampule of highly pure silica and dry; irradiate capsules at a thermal neutron density of $5x0^3 n/cm^{-2}/second^{-1}$	Instrumental NAA	0.0005 µg/L	>100	Jurgensen and Behne 1977
	Feed radiotracer ^{65}zinc; measure zinc activity in blood at 14 days	Tracer technique	NR	88	Watson et al. 1987

TABLE 6-1. Analytical Methods for Determining Zinc in Biological Materials *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood serum and red blood cells	Feed ^{68}zinc and ^{70}zinc and measure blood levels in a 24-hour sample and a sample taken immediately after zinc administration; wet ash sample; add APDC precipitant; dissolve precipitate in HNO_3; irradiate	Isotope tracer technique	NR	NR	Janghorbani et al. 1981
Blood	Feed $^{65}ZnCl_2$ orally; measure zinc blood levels and whole blood count	Radiotracer technique — whole blood count and blood level measurement	NR	88	Watson et al. 1987
Bloodstain	Place drop of blood on filter paper; cut away excess paper; optional dry ash; add HCl; shake	AAS	NR	NR	Fan et al. 1991
Thoracic aorta, lung, myocardium, spleen	Homogenize sample; complete wet ashing with HNO_3	AAS	NR	NR	Marks et al. 1972

TABLE 6-1. Analytical Methods for Determining Zinc in Biological Materials *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Feces	Give ^{67}Zn through diet; treat fecal samples with H_2O_2; prepare chelates	Isotope tracer technique	NR	>95 (^{67}Zn); 71 (^{70}Zn)	Johnson 1982
Feces	Feed ^{70}Zn, ^{68}Zn, and ^{64}Zn orally; homogenize sample; evaporate; ash; HNO_3 digestion; boil; evaporate; add HCl; transfer to anion exchange column; prepare eluate; irradiate	Isotope tracer technique, NAA	NR	NR	Ni et al. 1991
Bone	Acid digestion of dried bone ash with concentrated HNO_3; evaporate to dryness and add more concentrated HNO_3; remove silica residue by filtration; transfer samples to polyethylene bottles	AAS	NR	NR	Szpunar et al. 1978

TABLE 6-1. Analytical Methods for Determining Zinc in Biological Materials *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Hair	Digest clean sample in acid mixture	AAS	20 μg/g	NR	Wilhelm et al. 1991
Hair	Rinse sample with hexane; wet or dry ash with HNO_3	EDXRF	0.001 μg/L	NR	Folin et al. 1991
Hair	Rinse sample with hexane; wet or dry ash with HNO_3	AAS	0.001 μg/L	NR	Folin et al. 1991
Hair	Digest clean sample in acid mixture	ICP-AAS	NR	81–102	Takagi et al. 1988
Serum and plasma	Separate serum and plasma by centrifugation; keep stored in glass tubes at -20°C until analysis; thaw to room temperature prior to analysis	AAS	NR	NR	Shaw et al. 1982
Milk	Ash; lyophilize; wet-ash with HNO_3; add H_2O_2; dry; dissolve in HCl and NH_4Cl; extract with DDDC	ICP-MS	0.06 μg/sample	NR	Patterson et al. 1992

TABLE 6-1. Analytical Methods for Determining Zinc in Biological Materials *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Milk	Dilute sample with Triton X-100	AAS, electrothermal	0.052 μmol/L	86–106	Arnaud et al. 1991
Saliva	Lashley cup placed over one of the Stenson's ducts; secretion stimulated with lemon candies; discard first 5–10 mL; collect ≈120 mL	AAS	NR	NR	Langmyhr et al. 1979

AAS = atomic absorption spectroscopy; AES = atomic emission spectroscopy; APDC = ammonium pyrolidine dithiocarbamate; DDDC = diethylammonium diethyldithiocarbamate; EDXRF = energy dispersive x-ray fluorescence; FAAS = flame atomic absorption spectroscopy; FIA = flow injection analysis; GFAAS = graphite furnace atomic absorption spectroscopy; HCl = hydrogen chloride; $HClO_4$ = perchloric acid; HNO_3 = nitric acid; H_2O_2 = hydrogen peroxide; H_2SO_4 = sulfuric acid; ICP = inductively coupled argon plasma spectroscopy; MS = mass spectrometry; NAA = neutron activation analysis; NH_4Cl = ammonium chloride; NR = not reported; Zn = zinc; $ZnCl_2$ = zinc chloride

element composition with good precision, although its sensitivity is not always good (high ppm levels). Its principal limitation is that it requires analyzing each element separately (Szpunar et al. 1978). Graphite furnace AAS (GFAAS) has been used to determine zinc in human semen. Recovery (96-104%) was good, and preparation by microwave wet acid dissolution was more accurate than the standard water dilution method (Alvarado et al. 1991). AAS has also been used to determine zinc in bloodstains on filter paper. This method is accurate, reproducible, and acceptable for routine clinical testing using both dry ashing and direct extraction sample preparation (Fan et al. 1991). Electrothermal AAS is more sensitive than flame AAS (FAAS) and has been used to determine very low levels of zinc (detection limit, 0.052 μmol/L) in human milk (Arnaud et al. 1991). Zinc concentrations in liver have been accurately quantified by FAAS. Homogenization of tissue samples coupled with FAAS resulted in 100% recoveries, accuracies of 0-3%, and a detection limit of 0.04 mg/L (Luterotti et al. 1992).

Multi-elemental analysis has been used to detect zinc and other trace metals in biological fluids and tissues. For determination of metallic constituents in biological samples, such as liver, samples were digested with mixtures of different acids, volatile elements were distilled by selective distillation, and a cleanup step was performed using ion exchange chromatography prior to assay by neutron activation analysis (NAA) (Lievens et al. 1977). Recovery (98%) and precision (< 10% coefficient of variation [CV]) were excellent. Although the limit of detection for zinc was not reported, based on the reported results this method can detect levels ranging from the low- to the sub-ppm range (Lievens et al. 1977). The NAA technique has also been used to detect zinc in urine and blood samples. Jurgensen and Behne (1977) used the technique to measure human serum levels of trace elements including zinc. Recovery and precision for this method are very good. Sensitivity was not reported.

Inductively coupled plasma-atomic emission spectroscopy (ICP-AES) is used for multi-element determinations in blood and tissue samples. Detection in urine samples requires extraction of the metals with a polydithiocarbamate resin prior to digestion and analysis (NIOSH 1984a). Other satisfactory analytical methods include direct current plasma emission spectroscopy and determination by AAS, and inductively coupled argon plasma spectroscopy-mass spectrometry (ICP-MS) (Patterson et al. 1992; Shaw et al. 1982). Flow injection analysis (FIA) has been used to determine very low levels of zinc in muscle tissue. This method provides very high sensitivity,

low detection limits (3 ng/mL), good precision, and high selectivity at trace levels (Fernandez et al. 1992b).

The use of stable isotopes or tracers to study zinc absorption in humans with subsequent analysis by mass spectrometry has been reported in the literature. Analysis of fecal samples obtained 3 and 6 days after the administration of zinc-65 isotope in food showed that between 45% and 75% of zinc isotope was absorbed (Johnson 1982). The results indicated satisfactory detection of the zinc-67 isotope in human feces, while the zinc-70 isotope was not as detectable. Better precision and recovery were obtained for the zinc-67 isotope (2.4% CV; >95% recovery) than for the zinc-70 isotope (38% CV; 71% recovery). Sample detection limits were not reported. Total reported sample preparation time was <2 hours, and it took only 5-10 minutes to analyze each sample on the mass spectrometer.

A practical method, based on NAA, was developed for accurate measurement of the stable isotopes zinc-68 and zinc-70 in human plasma and red blood cells (Janghorbani et al. 1981). This method can provide an alternative to the use of radiolabeled zinc. It is more complex and time consuming than those used to measure radiolabeled zinc levels. As with any isotopic method, isotope exchange may invalidate calculation of net absorption, but this potential problem was not investigated. Precision was very good (<10%). Sensitivity and accuracy were not reported.

Radionuclide studies offer an additional method to investigate the factors that affect trace element absorption. Radioactivity emitted by the radionuclide was measured in blood 14 days after the oral ingestion of zinc-65 and compared with the amount of radioactivity emission determined by whole-body counting (Watson et al. 1987). The results indicated that, where whole-body counting facilities were not available, measurement of radioactivity emitted in blood was a reasonable alternative for the prediction of zinc absorption. Recovery for this method was adequate (88%); precision was acceptable (<17% CV). The limit of detection for zinc was not reported.

6.2 ENVIRONMENTAL SAMPLES

Table 6-2 lists the methods used for analyzing zinc in environmental samples.

TABLE 6-2. Analytical Methods for Determining Zinc in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collect air particulates on teflon filters; digest with HNO_3	NAA (nondestructive)	NR	NR	Zoller et al. 1974
Atmospheric aerosols	Collect sample on cellulose filter; digest with HNO_3; filter; dry; add HNO_3; adjust pH; add KNO_3	ASV	13.7 µg/L	NR	Casassas et al. 1991
Water and waste water	Acid digestion	AAS, direct aspiration	5 µg/L	99.3–111 at 60–310 µg/L	EPA 1979c (method 289.1)
Water and waste water		AAS, furnace technique	0.05 µg/L	NR	EPA 1979c (method 289.2)
Water	HNO_3 digestion; read samples at 213.9 nm	AAS, flame technique	NR	NR	AOAC 1984 (method 33.089)
Water	Mineralize sample in muffle furnace; dissolve in HNO_3	FIA	3 µg/L	NR	Fernandez et al. 1992b
Seawater	APDC-MIBK extraction	AAS	0.05 ppb	NR	Brooks et al. 1967

TABLE 6-2. Analytical Methods for Determining Zinc in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Seawater	Take a sample digest in the electrochemical cell; adjust pH; add chelating agent and aerate	Cathodic stripping voltammetry	$7x0^{-11}$ M	NR	van den Berg 1986
Crude oil	Digest sample with HNO_3; extract with MIBK or dilute with MIBK	AAS	0.8 μg/g	NR	Elson et al. 1981
Soil, solid waste, sludges	Acid digestion	ICP or flame AAS	2 μg/L (in solution)	102.5 at 80 μg/L	EPA 1986a (methods 6010 and 3050)
Soil, solid waste, sludges		AAS, direct aspiration0.005 μg/L (in solution)NREPA 1986a (method 7950)			
Soil	Extract with DTPA and NH_4HCO_3-DTPA	ICP	NR	NR	Boon and Soltanpour 1991
Plants	Digest samples with acids	AAS	NR	NR	AOAC 1984 (method 3.013)

TABLE 6-2. Analytical Methods for Determining Zinc in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Plants	Digest samples with acid; extract with dithiozone reagent and CCl_4; add HCl and CCl_4; read at 525 nm for mixed-color method and at 535 nm for single-color method	Mixed and single color methods — spectrophotometric analysis	NR	NR	AOAC 1984 (methods 3.054 and 3.061)
Food	Digest sample with acid mixtures; remove sulfide, nickel, and cobalt; add dithiozone and CCl_4; measure transmission at 540 nm	Colorimetric method	NR	NR	AOAC 1984 (method 25.168)
Food	Digest samples with acid mixtures	AAS	NR	NR	AOAC 1984 (method 25.175)
Food	Dry ash sample in muffle oven; dilute with HNO_3	FAAS; FES	0.24 μg/g	97–100	Morales-Rubio et al. 1992

TABLE 6-2. Analytical Methods for Determining Zinc in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Food	Clarify; de-gas; dilute with deionized water; add HNO_3 to solid samples	GFAAS	NR	90–113	Wagner et al. 1991
Food	Sample dependent; blend; lipophilize; grind; oven-dry; press into pellets	EDXRF	0.8 ppm	NR	Nielson et al. 1991
Shellfish	HNO_3 digestion in microwave; dilute with deionized water	FAAS	0.12 ppm	80	McCarthy and Ellis 1991
Natural waters	Acid digestion	AAS	0.005 ppm	NR	Fishman 1966

AAS = atomic absorption spectroscopy; APDC = ammonium pyrolidine dithiocarbamate; ASV = anodic stripping voltammetry; CCl_4 = carbon tetrachloride; DTPA = diethylenetriaminepentaacetic acid; EDXRF = energy dispersive x-ray fluorescence; FAAS = flame atomic absorption spectrometry; FES = flame emission spectrophotometry; FIA = flow-injection analysis; HCl = hydrochloric acid; $HN0_3$ = nitric acid; ICP = inductively coupled argon plasma spectroscopy; KNO_3 = potassium nitrite; MIBK = methyl isobutyl ketone; NAA = neutron activation analysis; NH_4HCO_3-DTPA = ammonium bicarbonate–diethylenetriaminepentaacetic acid; NR = not reported

6. ANALYTICAL METHODS

Variations of the AAS technique are commonly used to detect zinc levels in air, water, and soil samples, as well as in certain plant and food samples. Inductively coupled argon plasma spectroscopy (ICP) is a recommended test method for analyzing solid waste samples to measure zinc. Using either the ICP method or the AAS method, operators of hazardous waste management facilities can determine whether a given sample is hazardous, based on the level of zinc in a sample of solid waste leachate.

AAS has been used to determine zinc concentrations in natural waters (Fishman 1966). AAS is a rapid method of measuring zinc, with the detection limit (0.005 ppm) being somewhat better than those obtained using other methods. Recovery and precision data were not reported. A year later, Brooks et al. (1967) demonstrated a simple extraction system consisting of only two reagents, ammonium pyrollidine dithiocarbamate (APDC) and methyl isobutylketone (MIBK), with subsequent analysis by AAS to measure particulate and "soluble" zinc in seawater. Sensitivity was in the sub-ppm range, and precision was good (3% CV). Accuracy was not reported.

FAAS, coupled with microwave digestion and GFAAS, has been used to determine the concentration of zinc in food and shellfish samples. Limits of detection ranged from 0.12 to 0.24 ppm, with recoveries ranging from 80% to 113%. Precision and recovery using microwave digestion were comparable to traditional wet ashing and superior to dry ashing in shellfish samples (AOAC 1984; McCarthy and Ellis 1991; Morales-Rubio et al. 1992). GFAAS was also used to determine low levels of zinc in beer. Recovery (94-106%) and precision (4.2% CV) were excellent. Sensitivity was not reported (Wagner et al. 1991). Energy dispersive x-ray tluorescence (EDXRF) for multielement analysis has been used to detect zinc in dried food samples with better precision (and a detection limit of 0.8 ppm) than AAS methods (Nielson et al. 1991).

Cathodic stripping voltammetry, also known as adsorption voltammetry, has been used to detect various metal ions in a 10^{-l0} to 10^{-10} M range in seawater (van den Berg 1986). APDC was used as a chelating agent for zinc. Because of the great sensitivity and specificity of APDC for zinc, it can be detected directly in the unaltered sample. Recovery and precision data were not reported. Similarly, differential pulse cathodic stripping voltammetry (DPCSV) and differential pulse anodic stripping voltammetry (DPASV) after complexation with APDC have been used for determining zinc speciation at nanomolar concentrations in ocean waters (Donat and Bruland 1990). Recovery and precision data were not reported. Anodic stripping voltammetry (ASV) has been

used to detect zinc and other metal ions simultaneously at trace levels in atmospheric aerosols. This method is primarily used for small samples with very low concentrations of zinc. The limit of detection was 13.7 ng/L, and the recovery was not reported (Casassas et al. 1991).

AAS has been used to measure heavy metals, including zinc, in various oil samples collected at different stages of oil refining (Elson et al. 1981). These samples were prepared using three techniques (digestion, extraction, and dilution) prior to AAS analysis; recovery from crude oil was higher with wet digestion. Sensitivity for zinc was in the low-ppm range. Accuracy and precision were not reported.

An ion chromatographic method has been proposed for simultaneous determination of several elements including zinc in soil (Basta and Tabatabai 1990). In this method, after preliminary sample treatment, the metals are separated by ion chromatography, and the separated elements are quantitated by ultraviolet-visible detection of zinc-PAR (4-[2-pyridylazo] resorcinol) colored complexes. The limit of detection for zinc by this method is 5 ppb in soil extract. Precision was ≤2.5% CV. Accuracy was not reported. The concentration of zinc in soil was determined by ICP coupled with an ammonium bicarbonate-diethylenetriaminepentaacetic acid (NH_4,HCO_3,-DTPA) extraction procedure. (This method is superior to AAS and calorimetric methods because of the capacity for multielemental analysis.) This method can be used to screen soils for zinc. Limits of detection and recovery were not reported (Boon and Soltanpour 1991).

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of zinc is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of zinc.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be

interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. AAS is the most commonly used analytical method to determine zinc levels in plasma, bone, fingernails, hair, and other biological tissues and body fluids (Alvarado et al. 1991; Arnaud et al. 1991; Fan et al. 1991; Langmyhr et al. 1979; Luterotti et al. 1992; Marks et al. 1972; NIOSH 1984a; Shaw et al. 1982; Sohler et al. 1976; Szpunar et al. 1978; Wilhelm et al. 1991). This method generally is sensitive enough to measure background levels in the population and levels at which biological effects occur. The method is specific and precision is good. However, improved sensitivity and recovery data are needed in order to better evaluate the relationship between body and environmental exposure levels of zinc. Other methods that are specific for measuring zinc in biological fluids and tissues include NAA, ICP-AES, FIA, and isotope tracers techniques (Fernandez et al. 1992b; Janghorbani et al. 1981; Johnson 1982; Lievens et al. 1977; NIOSH 1984a; Watson et al. 1987). Sensitivity and/or recovery data for these methods are needed to more fully evaluate the reliability of these methods as predictors of environmental exposure.

Although several biomarkers for the effects of zinc have been identified (increased levels of serum amylases and lipase, non-iron responsive anemia, and decreased HDL cholesterol levels), these biomarkers of effect are not specific for zinc (Cotran et al. 1989; Suber 1989). Standard laboratory tests are available that can measure these biomarkers (Henry 1984). These methods are sensitive, accurate, and reliable enough to measure background levels in the population and levels at which biological effects occur. The development of methods for determining biomarkers of effect specific for zinc would be beneficial in assessing whether an individual has been exposed to zinc.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods of adequate sensitivity and specificity are available for determining levels of zinc in environmental media (AOAC 1984; Basta and Tabatabai 1990; Brooks et al. 1967; Casassas et al. 1991; Donat and Bruland 1990; Elson et al. 1981; EPA 19795 1986a; Fishman 1966; McCarthy

and Ellis 1991; Morales-Rubio et al. 1992: Nielson et al. 1991; van den Berg 1986; Wagner et al. 1991; Zoller et al. 1974). Some of these methods are precise and sensitive enough to measure background levels in the environment and levels at which health effects occur. These methods can distinguish between soluble zinc, insoluble zinc, and chelated zinc in water (Donat and Bruland 1990). Studies to obtain more information on the accuracy of these methods as well as improved sensitivity are needed to better assess the risk of exposure for these media. Research investigating the relationship between levels measured in air, water, and soil and observed health effects would increase our confidence in existing methods and/or indicate where improvements are needed.

6.3.2 On-going Studies

No on-going studies were located regarding techniques for measuring or detecting zinc in biological fluids, tissues, or environmental samples.